

Liraglutide Attenuates the Depressive- and Anxiety-like Behaviour in the Corticosterone Induced Depression Model Via Improving Hippocampal Neural Plasticity

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ABSTRACT

Recent studies indicate that metabolic disorders such as diabetes and obesity are a major risk factor of psychiatric diseases. This relationship opens the opportunity to develop new antidepressant drugs by repurposing antidiabetic drugs. Previous research has demonstrated that GLP-1 analogs are neuroprotective in several neurological disease models including Alzheimer's disease (AD), Parkinson's disease (PD), and stroke. In addition, the GLP-1 analog liraglutide has been shown to promote neurogenesis, which is seen to play important roles in memory formation and cognitive and emotional processing. However, whether liraglutide is an effective antidepressant remains unknown. Therefore, we tested this hypothesis in the depression model of chronic administration of corticosterone (CORT) in mice and treated the animals daily with liraglutide (5 or 20nmol/kg ip.) to assess its therapeutic potential as an antidepressant. Behavioral studies showed that liraglutide administration attenuated depressive- and anxiety- like behaviors in this depression mouse model, and attenuated the hyperactivity induced by the stress hormone. Additionally, liraglutide treatment protected synaptic plasticity and reversed the suppression of hippocampal long-term potentiation induced by CORT administration, demonstrating synaptic protective effects of liraglutide. We also found that liraglutide treatment increased the cell density of immature neurons in the subgranular dentate gyrus region of the hippocampus. In addition, liraglutide prevented the CORT induced impairments and simultaneously increased the level of phosphorylated GSK3 β in the hippocampus, which may be instrumental in the anti-depressant activity of liraglutide treatment. Taken together, liraglutide has the potential to act as a therapeutic treatment of depression.

KEY WORDS

Diabetes; Depression; Liraglutide; ACTH; Long-term Potentiation; Neurogenesis; Glucocorticoid Receptors; GSK3 β .

INTRODUCTION

Depression is one of the major causes of disability and a main health burden in the world. Emotional behaviors are influenced and controlled by the activity of the hippocampus, which is a brain region that shows high synaptic plasticity to enable animals to adapt to environmental challenges (LeDoux, 1993). Therefore, maintaining normal hippocampal neural function is one of the main targets in the therapeutic treatment of depression. Synaptic plasticity in the hippocampus plays critical roles in emotional memory and learning processes, which may be encoded in long-term potentiation (LTP) of synaptic plasticity in the hippocampus (Hölscher, 2003; LeDoux, 1993). Adult neurogenesis, the continuous process of neural stem cells (NSCs) dividing and developing into mature neurons in the adult brain, has been suggested to play a role in depression (Santarelli et al., 2003). In addition, most traditional antidepressant drugs promote neurogenesis in the adult hippocampus (Surget et al., 2008). Stress hormones such as corticosterone (CORT) can induce depression, and chronic CORT injection is an animal model of inducing a form of depression (Jalewa et al., 2014). CORT treatment also impairs hippocampal long-term potentiation (LTP), a cellular model of memory formation. This is an important pathophysiological feature in depression (Pavlidis et al., 1993). There are several molecular pathways that play a role in promoting hippocampal neurogenesis and LTP. In depression, stress induced hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis induces persistently elevated levels of glucocorticoids and stimulate hippocampal glucocorticoid receptors (GR), which subsequently results in the suppressed proliferation of adult NSCs (Egeland et al., 2015). In addition, previous studies show that high corticosterone levels cause a suppression in LTP in the hippocampus (Pavlidis et al., 1996). Furthermore, enhanced GSK3 β activity has been observed in depression, and appears to play a role in the impairment of neural precursor proliferation by the GR agonist dexamethasone (Boku et al., 2009). The increase of GSK3 β activity was also found to reduce LTP (Peineau et al., 2007). Moreover, inhibiting GSK3 β activity was found to improve hippocampus-dependent learning and rescued impaired adult hippocampal neurogenesis in a mouse model of fragile X syndrome (Guo et al., 2012). In animal models of depression, reducing GSK3 β activity with lithium improved stress-induced changes in depression-like behavior (Silva et al., 2008).

Previous studies indicate that metabolic disorders like obesity and diabetes are one of the major risk factors of psychiatric disorders including depression (Auxier et al., 2012; Khater and Omar, 2017). Indeed, the successful use of antidiabetic agents in neurological diseases

gave rise to the idea that the same drugs may have beneficial effects on depression. GLP-1 is an incretin hormone and plays a role in the regulation of blood glucose levels and insulin sensitivity (Kahn et al., 2006). GLP-1 analogs like liraglutide have been in use as antidiabetic agents for many years (Tran et al., 2017). Several studies that clearly show beneficial actions in mouse models of diabetes-obesity (Porter et al., 2013). In addition, previous studies demonstrated that liraglutide has neuroprotective effects in neurological disease models such as Alzheimer's disease (AD), Parkinson's disease (PD) as well as stroke (Han et al., 2013a; Liu et al., 2015; McClean and Holscher, 2014; Sato et al., 2013). However, whether liraglutide has antidepressive effects in the chronic elevated (Hypothalamus-Pituitary-Adrenal) HPA axis -induced depression model and whether liraglutide can improve the impairment of hippocampal neural plasticity associated with depression has not been reported previously. The enhancing effects of liraglutide on hippocampal plasticity in wild type rats or transgenic mouse model of Alzheimer's disease has been shown before (Han et al., 2013b; McClean et al., 2010; McClean and Holscher, 2014). To further investigate the effects of liraglutide on CORT induced synaptic impairment, we performed LTP recording in the hippocampal area CA1. Additionally, it is not known if liraglutide can regulate GSK3 β activity or the beta-catenin pathway in an animal model of depression. Therefore, we used the chronic administration of corticosterone (CORT) depression mouse model (Jalewa et al., 2014) to test this hypothesis and treated the animals with liraglutide at different doses. As a positive control, we also tested the effects of the classic selective serotonin re-uptake inhibitor (SSRI) fluoxetine to compare to the effects of liraglutide.

MATERIAL AND METHODS

Animals

Adult male C57BL/6N mice (8 weeks) were obtained from the animal center of Xiangya Medical School, Central South University. All animal treatments and experimental procedures were performed in accordance to National Institutes of Health (NIH) guideline (National Institutes of Health Publications, No. 80-23, revised 1978). The depression model was established by oral corticosterone (CORT) chronic administration. In brief, mice were treated with oral administration of CORT for 30 days (35 μ g/ml/d, equivalent to 5 mg/kg/d) see (Siopi et al., 2016). To reach the goal in investigating the treatment effect of liraglutide,

from the 15th day of the treatment with CORT, mice received intraperitoneal injection (IP) of different dosage of liraglutide (5, 20 nmol/kg) purchased from GL Biochem Ltd. Shanghai, China, or fluoxetine (FLX, 18 mg/kg/d; Sigma-Aldrich, US) or saline. The dosage of liraglutide was chosen, because it showed neuroprotective effects previously (Parthsarathy and Holscher, 2013).

Behavioral Tests

Tail suspension test

The tail suspension test (TST) was conducted at 09:00am~10:00am. During the test, mice were suspended by the tip of their tails for 6 min. The distance from the nose of mice to the floor was 25cm. Mobility time (struggling exclude the movement of paws only) of mice were recorded for analyzing depressive mood (Can et al., 2012). The activity of mice was video recorded, and the mobility time was recorded by an observer blind to treatment.

Forced swimming test

The forced swimming test was conducted on the same day of TST at 17:00~18:00pm. Mice were put into a cylinder water tank (30 cm height × 20 cm diameters) with water temperature at 23~25°C for 6min. The mobility time within last 4 min (define the first 2 min as habituation) including struggling and free swim time were recorded for analyzing. The movements of mice was video recorded, and the mobility time was recorded by an observer blind to treatment.

Open field test for anxiety

Mice were put into an open field with the size of 43cm × 43cm for 10min. Mice could freely move in the arena. Duration of the animal traveled in the central area (20cm square region in center of the arena) was recorded to analyze the anxiety level. The Open field was cleaned with 75% ethanol before each test. The time of mice to spent in the central region was recorded by an observer blind to treatment.

Elevated Plus Maze

Elevated plus maze (EPM) was conducted on a plus-cross-shaped apparatus that was elevated 58 cm above the floor and comprised two open and two closed arms (30×6 cm) that extended from a central platform (7×7 cm). Mice were free to move on the maze for 10min.

The time spent and entries in center, open arm, and closed arm zones were calculated (Siopi et al., 2016). The time of mice to spent in the both open arms and closed arms region were recorded by an observer blind to treatment.

Hippocampal Slice Electrophysiology Recording

Brains were quickly removed and submerged in ice-cold oxygenated sucrose-replaced artificial cerebrospinal fluid (ACSF) cutting solution containing (in mM) 206 sucrose, 2 KCl, 2 MgSO₄, 1.25 NaH₂PO₄, 1 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 10 d-glucose, pH 7.4, 315mOsm. Transverse slices (300 μm thickness) from the middle portion of each hippocampus were cut with the vibroslicer (VT1200 S, Leica, Germany). After dissection, slices were incubated in ACSF that contained the following (in mM): 124 NaCl, 2 KCl, 2 MgSO₄, 1.25 NaH₂PO₄, 2.5 CaCl₂, 26 NaHCO₃, 10 D-glucose (pH 7.4), 310 mOsm, in which they could recover for at least 90 min before recording. A single slice was then transferred to the recording chamber and submerged beneath continuously perfuse of ACSF that had been saturated with 95% O₂ and 5% CO₂.

A bipolar stimulating electrode (FHC Inc., Bowdoin, ME) was placed in the Schaffer collaterals to deliver test and conditioning stimuli. A borosilicate glass recording electrode filled with ACSF was positioned in stratum radiatum of CA1, 200~300 μm from the stimulating electrode. fEPSP in the CA1 region were induced with an intensity that elicited a fEPSP amplitude 40%–50% of maximum. Test responses were recorded for 30–60 min prior to beginning the experiment to assure stability of the response. Once a stable test response was attained, experimental treatments were added to the 10 ml ACSF perfusate, and a baseline was recorded for an additional 30 min. To induce LTP, high frequency stimulation (HFS) including two consecutive trains (1s) of stimuli at 100 Hz separated by 20 s were applied to the slices, a protocol that induced LTP lasting approximately 1h. The field potentials were amplified 100× using an Axon Instruments 200B amplifier and digitized with Digidata 1322A. Data were sampled at 10 kHz and filtered at 2 kHz. Traces were obtained by pClamp 9.2 and analyzed using the Clampfit 9.2 program. LTP values reported throughout were measured at 60 min after the conditioning stimulus unless stated otherwise.

Immunofluorescence

After LTP recording, the hippocampal slices were immediately fixed with 4% paraformaldehyde (PFA) for 24 h. Then, the slices were dehydrated in 30% of sucrose solution for 2 days, and embedded with frozen embedding medium OCT (Leica Inc., Germany). Coronal-brain sections (thickness 40 μm) were prepared with freezing microtome (Leica Inc., Germany). Before IHC, antigen was retrieved with pH6.0 citrate acid buffer heated in micro-oven for 10 min (high power). Then sections were washed with PBS (0.4% TritonX-100) for 3 times (15 min per time) and blocked with 5% goat serum (Zhongshan Jinqiao Inc., China) for 30 min. Primary doublecortin (DCX) antibody (Rabbit-anti-DCX, CELL SIGNALING TECHNOLOGY, 1:500) was incubated with the brain section overnight at 4°C. Secondary fluorescent antibodies (Goat-anti-Rabbit-Alexa 488, Thermo Fisher, 1:800) was incubated in room temperature for 2 h after washing the primary antibodies with PBS for 3 times (5 min per time). Finally, sections were incubated with DAPI (4',6-diamidino-2-phenylindole) solution for 10 min. DCX+ NPCs in hippocampal DG were observed with the confocal microscope (Zeiss, LSM 700, German). Cell density were quantitatively analyzed with the ImageJ software (<https://imagej.nih.gov/ij/>).

ELISA

Plasma preparation was performed at 09:00am-10:00am. For baseline corticosterone detection, mice were normally raised before blood collection. Blood was collected from the tail vein before stress treatment. Then, mice underwent the acute stress treatment by restraining mice into a plastic tube with 11 air holes for 30min. Afterward, blood was collected again. Plasma was immediately prepared by centrifugation of the blood at 4000rpm (1800g) at 4°C for 15min. ACTH ELISA kit (Enzo Life Science, US) was employed for plasma tests of ACTH levels. Samples were all diluted 40 times following the instruction of ELISA kit beforehand. A plate reader (Bio-rad 680, US) was used for detecting the OD value of each well.

Westernblot

Hippocampal slices were collected and protein lysate was prepared with RIPA lysis buffer with 1% of phosphatase inhibitor (Beyotime Inc., China). The BCA method was applied to quantitate the protein concentration of each sample. 40 μg of the protein was loaded in each well of the gel (10% SDS-PAGE separation gel). SDS-PAGE electrophoresis was performed

to separate the proteins by the molecular size. Transfer of proteins onto 0.45 μ m PVDF membrane was done using the semi-dry transfer method. The membrane was blocked with 0.1% BSA for 1h at room temperature. Primary antibodies (Rabbit anti-DCX, 1:1000; Rabbit anti-phospho-GR (S211), 1:1000; Rabbit anti-GR, 1:1000; Rabbit anti- β -catenin, 1:1000; Rabbit anti-phospho-GSK3- β (S9); Rabbit anti- GSK3- β ; and Rabbit anti-GAPDH, 1:2000; Cell Signaling Technology, US) were incubated with the membrane at 4°C overnight. The membrane was washed with TBST buffer for 3 times (15min each time). The secondary antibody (Goat anti-rabbit HRP, CELL SIGNALING TECHNOLOGY, 1:5000) was incubated with the membrane for 1h at room temperature. Protein bands were visualized with enhanced chemiluminescent (ECL, Pierce, Rockford, IL, USA) and analyzed by the imaging system Quantity One 4.31 (Bio-Rad, Hercules, CA, USA).

Statistics

All results were presented as mean \pm standard error and displayed with the graphic software Prism 6 (GraphPad, GraphPad Software Inc., La Jolla, CA, USA). The software SPSS 16.0 (GraphPad) was used for statistical analysis. A two-way ANOVA was used for analyzing data related to time changes (LTP recording). The rest of analysis was conducted by one-way ANOVA. Statistically significance was classed as $p < 0.05$. Mark the significance comparing through *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$ compared with control; #: $p < 0.05$, ##: $p < 0.01$; ###: $p < 0.001$ compared with model.

RESULTS

Liraglutide attenuates the depressive-/anxiety-like behaviors induced by CORT

We conducted the behavioral tests to assess whether liraglutide could ameliorate the depression- associated symptoms induced by CORT administration. After 30 days of CORT administration and 15 days liraglutide/fluoxetine treatment, the tail suspension test (TST) and forced swim test (FST) were performed to evaluate any depressive behavior (Fig 1 A, $n=12$ per group). CORT administration resulted in decreased mobility in the TST and FST (Fig 1 B, C; one-way ANOVA, $p < 0.001$), which indicates that depression had been induced by chronic stress. No significant effects were observed with the lower dosage of liraglutide to the depressive behavior (Fig 1 B, C; one-way ANOVA, $p=0.543$ in TST; $p=0.784$ in FST), but

the high dosage of liraglutide (20nmol/kg/d) improved the mobility time in TST and FST, indicating the antidepressant effects of liraglutide (Fig 1 B, C; one-way ANOVA, $p < 0.001$ in TST; $p < 0.01$ in FST). As the positive control, antidepressant fluoxetine (FLX) also attenuated the depression-like behaviors of the mice in the TST and FST (Fig 1 B, C; one-way ANOVA, $p < 0.001$ in TST; $p = 0.0011$ in FST). Elevated plus maze (EPS) as well as open field test were then performed to assess the anxiety-like behavior of the mice and the effects of liraglutide as an anxiolytic. During EPS, CORT administration caused a shortening of exploration time in the open arm (Fig 1 D, one-way ANOVA, $p < 0.001$). Additionally, CORT administration also decreased the tendency of mice to explore the central region of open field (Fig 1 E, one-way ANOVA, $p < 0.001$). Those results document the anxiety-like behavior induced by chronic CORT administration. Unlike the positive control FLX (Fig 1 D, one-way ANOVA, $p < 0.001$), both low and high dosage of liraglutide did not increase the time of the mice spent in open arm (Fig 1 D, one-way ANOVA, $p = 0.1413$ for low dosage; $p = 0.1499$ for high dosage). However, both dosages of liraglutide prolonged the exploration time in central area of open field (Fig 1 E, one-way ANOVA, $p = 0.0485$ for low dosage; $p = 0.029$ for high dosage), which resemble the effects of FLX (Fig 1 E, one-way ANOVA, $p = 0.0342$). Taken together, liraglutide treatment could attenuate most depressive- and anxiety-like behaviors induced by chronic stress.

Liraglutide reduces the secretion of the stress hormone ACTH under stress stimulation

To investigate the effects of liraglutide on the stress response of the depression model, we tested the levels of the stress hormone adrenocorticotropic hormone (ACTH) ($n = 6$ per group, Fig 2 A). The level of ACTH in plasma were tested before and after stress treatment to test the changes of the response to stress. The result demonstrates that CORT group mice showed a significantly increased production of ACTH under stress (Fig 2 A, B, two-way ANOVA, $p < 0.0001$ compared with control). Compared with the CORT group, liraglutide at both dosages ameliorated the level of the stress response by down-regulating the ACTH levels when mice underwent the restraint stress treatment (Fig 2 B, two-way ANOVA, $p < 0.0001$ compare with CORT group). In addition, the positive control FLX also showed the same effects (Fig 2, A, B, two-way ANOVA, $p < 0.0001$ compare with CORT group).

Liraglutide treatment enhanced synaptic plasticity in hippocampal area CA1

LTP levels were analyzed in 5 groups (n=6 per group, Fig 3 A). We confirmed that CORT administration resulted in decreased LTP induction and maintenance compared with control (Fig 3 B, two-way ANOVA, $p < 0.001$), which was reflected by decreased fEPSPs at different time points after high-frequency stimulation (HFS) (Fig 3 C, two-way ANOVA; $p < 0.001$ at 0min; $p = 0.0014$ at 30min; $p = 0.003$ at 60min). Low dosage of liraglutide treatment did not have an effect compared with CORT group (Fig 3 B two-way ANOVA, $p = 0.6015$), but high dosage liraglutide significantly improved the strength of LTP that had been impaired by CORT administration (Fig 2 B two-way ANOVA, $p < 0.001$). Compared with the CORT group, high dosage of liraglutide treatment increased fEPSPs as measured at 3 time points after HFS (Fig 3 C, two-way ANOVA; $p < 0.001$ at 0min; $p = 0.0377$ at 30min; $p = 0.0069$ at 60min). Interestingly, FLX treatment did not show an increase in fEPSPs at 0min (Fig 3 C, two-way ANOVA; $p = 0.0092$ at 0min; $p = 0.9955$ at 30min; $p = 0.9596$ at 60min). Although a two-way ANOVA test showed a significant improvement of LTP by FLX in area CA1 (Fig 3 B, two-way ANOVA, $p < 0.001$), the effects of FLX on LTP maintenance was inferior compared to liraglutide. Taken together, liraglutide attenuates the chronic stress induced synaptic impairment, which may contribute to its protective effect in depression.

Liraglutide promotes adult neurogenesis in the dentate gyrus area of the hippocampus

We analyzed the expression level of hippocampal doublecortin (DCX), which acts as the marker of the immature developing neurons (n=6 per group, Fig 4). The results indicate that chronic treatment of CORT resulted in decreased levels of DCX in the hippocampus (Fig 4 A, B, one-way ANOVA, CORT vs Control: $p < 0.0001$). Compared with the CORT group, 20nm/kg liraglutide treatment showed increased levels of DCX in the hippocampus (Fig 4 A, B, one-way ANOVA, CORT vs 20nm/kg: $p = 0.149$). This effect was also observed in the positive control FLX treatment group (Fig 4 A, B, one-way ANOVA, CORT vs FLX: $p = 0.0067$). CORT treatment caused a decreased density of DCX+ neurons in DG (Fig 4 C, D, one-way ANOVA, CORT vs Control: $p < 0.0001$). Liraglutide treatment at the high dosage (20nm/kg) significantly increased the density of DCX+ immature neurons in comparison with CORT (Fig 4 C, D, one-way ANOVA, CORT vs 20nm/kg: $p = 0.0457$). This effect was also found in the positive control FLX treated group (Fig 4 C, D, one-way ANOVA, CORT vs FLX: $p = 0.0307$). Taken together, the results suggest that liraglutide can enhance adult hippocampal neurogenesis, which is impaired in depression.

Liraglutide reduces the over-activation of GSK3 β in depression

We tested the expression level of GSK3 β as well as its phosphorylation at the Ser9 site (Fig 5 A). The results show that there was no significant change of the total expression level of GSK3 β in the hippocampus among different groups (Fig 5 A, B, n=6, one-way ANOVA, p=0.3904). When comparing the phosphorylation level of GSK3 β at Ser9 site, the CORT group presented a down- regulation compared to control (Fig 5 A, C, n=6, one-way ANOVA, CORT vs Control: p<0.0001). In addition, treatment of liraglutide at the high dosage (20nm/kg) increased the phosphorylation level of GSK3 β at Ser9 in comparison with the CORT group (Fig 5 A, C, n=6, one-way ANOVA, 20nm/kg vs CORT: p=0.0312). The lower dosage of liraglutide (5nm/kg) did not reach statistical significance (Fig 5 A, C, n=6, one-way ANOVA, 5nm/kg vs CORT: p=0.2378). Moreover, the positive control FLX did not significantly reduce the activity of GSK3 β by increasing the phosphorylation level at Ser9 site (Fig 5 A, C, n=6, one-way ANOVA, FLX vs CORT: p=0.1381).

DISCUSSION

Our study showed that the GLP-1 drug liraglutide has beneficial effects on CORT induced depressive- and anxiety- like symptoms. In addition to the observed protective effects in neurodegenerative diseases, liraglutide may act as an antidepressant drug. The behavioral studies indicate that liraglutide treatment attenuates the chronic CORT induced depressive-/anxiety- behaviors. The performance of the mice treated with the higher dose of liraglutide is as good as the FLX treated animals. Therefore, the antidepressant effects shown here is promising and deserves further research. Liraglutide also normalized the ACTH levels after stress treatment, attenuating depressive-/anxiety- like behaviors and regulating the stress endocrine system.

LTP of synaptic activity in the hippocampus and adult neurogenesis have been implied to play a key role in memory formation and hippocampal-dependent behaviors (Hawkins et al., 1998; Stuchlik, 2014). Impaired hippocampal functions, especially decreased neural plasticity and LTP, contributes to the development of depression (Borsini and Zunszain, 2016; Dina et al., 2017). In current study, we found that liraglutide treatment normalized LTP in the hippocampus after CORT treatment, and promoted adult neurogenesis in hippocampus that had been impaired by CORT (Fig 3~4). This result suggests that the improvement of LTP as well as neurogenesis are correlated with the antidepressant effects of liraglutide. These results

confirm previous research in AD animal models that showed improved neurogenesis in the dentate gyrus as well as LTP induction after liraglutide treatment (Hamilton et al., 2011; McClean et al., 2011a). Collectively, the anti-depressant activity of liraglutide may be driven by regulating the neural plasticity in the adult hippocampus.

GSK3 β signaling is involved in the regulation of glucose metabolism (Summers et al., 1999). Since metabolism disorders are one of the important risk factors to neurological diseases, metabolic factors could also play fundamental roles in the development of neurological or psychiatric diseases. Over-activity of GSK3 β is also seen to play a role in depression-induced neural dysfunctions (Costemale-Lacoste et al., 2016), and GSK3 β is a target for the effects of anti-depression treatments (Zunzain et al., 2013). Previous studies showed that the natural compound geniposide, a GLP-1 receptor agonist, could reverse the over-activation of GSK3 β in a rat model of sporadic Alzheimer's disease (Gao et al., 2014). However, whether liraglutide could attenuate over-activated GSK3 β in a depression model had never been shown before. Here we demonstrate for the first time that liraglutide can attenuate the activity of GSK3 β which was elevated by CORT treatment. This result indicates that regulating GSK3 β activity plays a role in the neuroprotective effects of liraglutide in various neurological and psychiatric pathophysiologies.

When comparing the effects of liraglutide with those of the standard antidepressant drug FLX, we found that both drugs show similar improvements in key biomarkers that were measured in our study. However, in the LTP study of synaptic activity in the hippocampus, FLX showed no improvement, while liraglutide clearly did. This indicates that the mode of action is different for both drugs on some extent. Moreover, based on the different target that liraglutide does not improve the neural functions by directly elevating certain kinds of neurotransmitters as traditional antidepressants like fluoxetine, it could be more suitable to get rid of other symptoms induced by depression including insomnia, anorexia or even avoid the side effects induced by fluoxetine like obesity and addiction. Besides liraglutide, other GLP-1 medications like DPP4 inhibitors, which have shown to perform therapeutic effects in Alzheimer's disease models, might also have the potential as an antidepressant (D'Amico et al., 2010; Gault et al., 2015). Thus, the different mechanism between anti-diabetic drugs and fluoxetine is worthy of further research.

Some limitations cannot be avoided in this study. For example, the model we employed here cannot mimic the unpredictable stress-induced type of depression. But based on the theory of HPA axis hyperactivation, it is a good model to use for developing antidepressants. On the other hand, a treatment of liraglutide on control mice should be added to assess the side

effects of the drug. However, based on previous reports, chronic treatment of liraglutide did not affect the cognitive functions in wild type mice, so we did not include a drug treated control group in our study (McClellan et al., 2011b).

Taken together, the anti-diabetic drug liraglutide has the potential to act as a therapeutic treatment of depression. The underlying mechanisms include promoting hippocampal LTP and adult neurogenesis in a GSK3 β dependent mechanism. The drug is widely used and is safe to take for people who do not have diabetes. Currently, a clinical trial is ongoing that tests the effects of liraglutide in non-diabetic Alzheimer's patients (Hölscher, 2016). Further research is needed to compare GLP-1 mimetics with currently available treatments for depression and to analyze the underlying biochemical mechanisms in more detail.

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All authors declare no conflict of interest.

REFERENCES

- Auxier, A., C. Runyan, D. Mullin, T. Mendenhall, J. Young, and R. Kessler. 2012. Behavioral health referrals and treatment initiation rates in integrated primary care: a Collaborative Care Research Network study. *Transl Behav Med.* 2:337-344.
- Boku, S., S. Nakagawa, T. Masuda, H. Nishikawa, A. Kato, Y. Kitaichi, T. Inoue, and T. Koyama. 2009. Glucocorticoids and lithium reciprocally regulate the proliferation of adult dentate gyrus-derived neural precursor cells through GSK-3 β and beta-catenin/TCF pathway. *Neuropsychopharmacology.* 34:805-815.
- Borsini, A., and P.A. Zunszain. 2016. Advances in Stem Cells Biology: New Approaches to Understand Depression. *In Stem Cells in Neuroendocrinology.* D. Pfaff and Y. Christen, editors, Cham (CH).
- Can, A., D.T. Dao, C.E. Terrillion, S.C. Piantadosi, S. Bhat, and T.D. Gould. 2012. The tail suspension test. *Journal of visualized experiments : JoVE:*e3769.
- Costemale-Lacoste, J.F., J.P. Guilloux, and R. Gaillard. 2016. The role of GSK-3 in treatment-resistant depression and links with the pharmacological effects of lithium and ketamine: A review of the literature. *Encephale.* 42:156-164.
- D'Amico, M., C. Di Filippo, R. Marfella, A.M. Abbatecola, F. Ferraraccio, F. Rossi, and G. Paolisso. 2010. Long-term inhibition of dipeptidyl peptidase-4 in Alzheimer's prone mice. *Experimental gerontology.* 45:202-207.

- Dina, P., E. Castren, and T. Taira. 2017. Chronic fluoxetine administration enhances synaptic plasticity and increases functional dynamics in hippocampal CA3-CA1 synapses. *Neuropharmacology*.
- Egeland, M., P.A. Zunszain, and C.M. Pariante. 2015. Molecular mechanisms in the regulation of adult neurogenesis during stress. *Nat Rev Neurosci*. 16:189-200.
- Gao, C., Y. Liu, Y. Jiang, J. Ding, and L. Li. 2014. Geniposide ameliorates learning memory deficits, reduces tau phosphorylation and decreases apoptosis via GSK3beta pathway in streptozotocin-induced alzheimer rat model. *Brain pathology*. 24:261-269.
- Gault, V.A., R. Lennox, and P.R. Flatt. 2015. Sitagliptin, a dipeptidyl peptidase-4 inhibitor, improves recognition memory, oxidative stress and hippocampal neurogenesis and upregulates key genes involved in cognitive decline. *Diabetes, obesity & metabolism*. 17:403-413.
- Guo, W., A.C. Murthy, L. Zhang, E.B. Johnson, E.G. Schaller, A.M. Allan, and X. Zhao. 2012. Inhibition of GSK3beta improves hippocampus-dependent learning and rescues neurogenesis in a mouse model of fragile X syndrome. *Human molecular genetics*. 21:681-691.
- Hamilton, A., S. Patterson, D. Porter, V.A. Gault, and C. Holscher. 2011. Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. *Journal of neuroscience research*. 89:481-489.
- Han, W.-N., C. Holscher, L. Yuan, W. Yang, X.-h. Wang, M.-N. Wu, and J.-S. Qi. 2013a. Liraglutide protects against β -amyloid induced impairment of spatial learning and memory in rats. *Neurobiology of aging*. 32:576-588.
- Han, W.N., C. Holscher, L. Yuan, W. Yang, X.H. Wang, M.N. Wu, and J.S. Qi. 2013b. Liraglutide protects against amyloid-beta protein-induced impairment of spatial learning and memory in rats. *Neurobiology of aging*. 34:576-588.
- Hawkins, R.D., H. Son, and O. Arancio. 1998. Nitric oxide as a retrograde messenger during long-term potentiation in hippocampus. *Prog Brain Res*. 118:155-172.
- Hölscher, C. 2003. Time, space, and hippocampal functions. *Reviews in the neurosciences*. 14:253-284.
- Hölscher, C. 2016. GLP-1 and GIP analogues as novel treatments for Alzheimer's and Parkinson's disease. *Cardiovasc Endocrinol*. 5:93-98.
- Jalewa, J., K. Wong-Lin, T.M. McGinnity, G. Prasad, and C. Holscher. 2014. Increased number of orexin/hypocretin neurons with high and prolonged external stress-induced depression. *Behavioural brain research*. 272:196-204.
- Kahn, S.E., R.L. Hull, and K.M. Utzschneider. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 444:840-846.
- Khater, D., and M. Omar. 2017. Frequency and risk factors of depression in type 1 diabetes in a developing country. *J Pediatr Endocrinol Metab*.
- LeDoux, J.E. 1993. Emotional memory systems in the brain. *Behavioural brain research*. 58:69-79.
- Liu, W., J. Jalewa, M. Sharma, G. Li, L. Li, and C. Hölscher. 2015. Neuroprotective effects of lixisenatide and liraglutide in the MPTP mouse model of Parkinson's disease. *Neuroscience*. 303:42-50.
- McClean, P., V. Parthasarathy, E. Faivre, and C. Hölscher. 2011a. The diabetes drug Liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 31:6587-6594.
- McClean, P.L., V.A. Gault, P. Harriott, and C. Holscher. 2010. Glucagon-like peptide-1 analogues enhance synaptic plasticity in the brain: A link between diabetes and Alzheimer's disease. *European journal of pharmacology*. 630:158-162.
- McClean, P.L., and C. Holscher. 2014. Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. *Neuropharmacology*. 76 Pt A:57-67.
- McClean, P.L., V. Parthasarathy, E. Faivre, and C. Holscher. 2011b. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 31:6587-6594.
- Parthasarathy, V., and C. Holscher. 2013. The type 2 diabetes drug liraglutide reduces chronic inflammation induced by irradiation in the mouse brain. *European journal of pharmacology*. 700:42-50.
- Pavlidis, C., S. Ogawa, A. Kimura, and B.S. McEwen. 1996. Role of adrenal steroid mineralocorticoid and glucocorticoid receptors in long-term potentiation in the CA1 field of hippocampal slices.

Brain research. 738:229-235.

- Pavlidis, C., Y. Watanabe, and B.S. McEwen. 1993. Effects of glucocorticoids on hippocampal long-term potentiation. *Hippocampus.* 3:183-192.
- Peineau, S., C. Taghibiglou, C. Bradley, T.P. Wong, L. Liu, J. Lu, E. Lo, D. Wu, E. Saule, T. Bouschet, P. Matthews, J.T. Isaac, Z.A. Bortolotto, Y.T. Wang, and G.L. Collingridge. 2007. LTP inhibits LTD in the hippocampus via regulation of GSK3beta. *Neuron.* 53:703-717.
- Porter, W.D., P.R. Flatt, C. Holscher, and V.A. Gault. 2013. Liraglutide improves hippocampal synaptic plasticity associated with increased expression of Mash1 in ob/ob mice. *Int J Obes (Lond).* 37:678-684.
- Santarelli, L., M. Saxe, C. Gross, A. Surget, F. Battaglia, S. Dulawa, N. Weisstaub, J. Lee, R. Duman, O. Arancio, C. Belzung, and R. Hen. 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science.* 301:805-809.
- Sato, K., M. Kameda, T. Yasuhara, T. Agari, T. Baba, F. Wang, A. Shinko, T. Wakamori, A. Toyoshima, H. Takeuchi, T. Sasaki, S. Sasada, A. Kondo, C. Borlongan, M. Matsumae, and I. dDate. 2013. Neuroprotective effects of liraglutide for stroke model of rats. *International journal of molecular sciences.* 14:21513-21524.
- Silva, R., A.R. Mesquita, J. Bessa, J.C. Sousa, I. Sotiropoulos, P. Leao, O.F. Almeida, and N. Sousa. 2008. Lithium blocks stress-induced changes in depressive-like behavior and hippocampal cell fate: the role of glycogen-synthase-kinase-3beta. *Neuroscience.* 152:656-669.
- Siopi, E., M. Denizet, M.M. Gabellec, F. de Chaumont, J.C. Olivo-Marin, J.P. Guilloux, P.M. Lledo, and F. Lazarini. 2016. Anxiety- and Depression-Like States Lead to Pronounced Olfactory Deficits and Impaired Adult Neurogenesis in Mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 36:518-531.
- Stuchlik, A. 2014. Dynamic learning and memory, synaptic plasticity and neurogenesis: an update. *Front Behav Neurosci.* 8:106.
- Summers, S.A., A.W. Kao, A.D. Kohn, G.S. Backus, R.A. Roth, J.E. Pessin, and M.J. Birnbaum. 1999. The role of glycogen synthase kinase 3beta in insulin-stimulated glucose metabolism. *The Journal of biological chemistry.* 274:17934-17940.
- Surget, A., M. Saxe, S. Leman, Y. Ibarguen-Vargas, S. Chalon, G. Griebel, R. Hen, and C. Belzung. 2008. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol Psychiatry.* 64:293-301.
- Tran, K.L., Y.I. Park, S. Pandya, N.J. Muliylil, B.D. Jensen, K. Huynh, and Q.T. Nguyen. 2017. Overview of Glucagon-Like Peptide-1 Receptor Agonists for the Treatment of Patients with Type 2 Diabetes. *Am Health Drug Benefits.* 10:178-188.
- Zunszain, P.A., M.A. Horowitz, A. Cattaneo, M.M. Lupi, and C.M. Pariante. 2013. Ketamine: synaptogenesis, immunomodulation and glycogen synthase kinase-3 as underlying mechanisms of its antidepressant properties. *Mol Psychiatry.* 18:1236-1241.

FIGURES

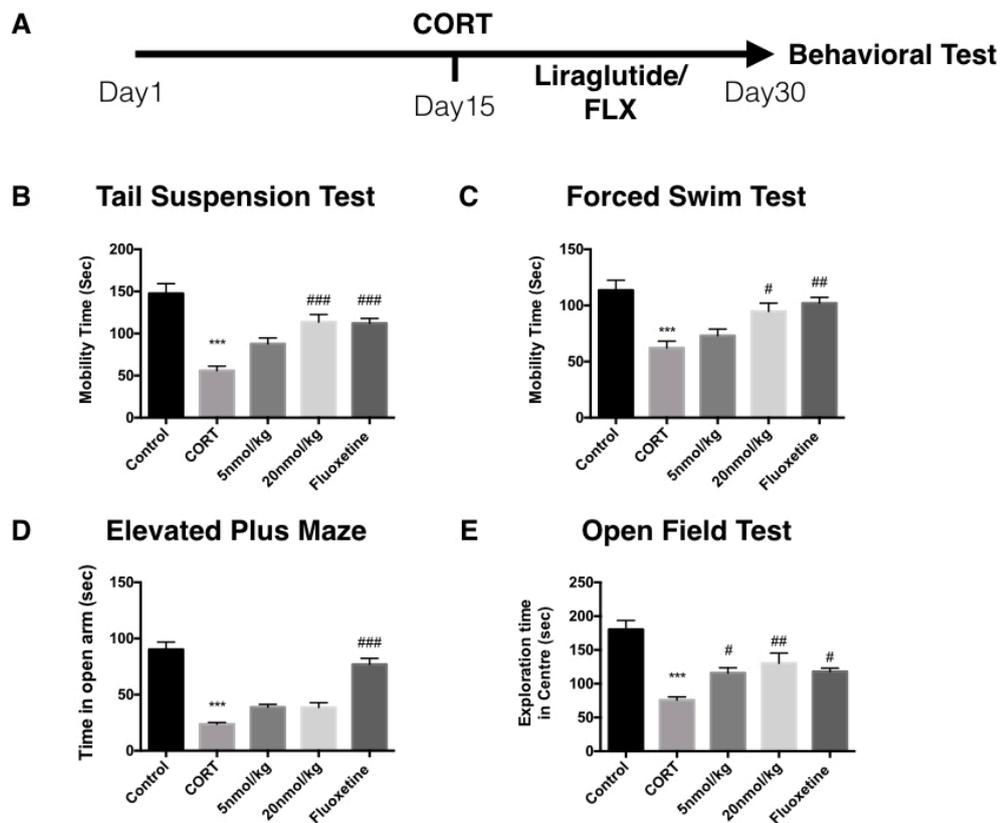


Figure 1: Liraglutide showed protective effects in the corticosterone (CORT) induced depression model in behavioral tasks

(A) Experimental procedure of the study. CORT was administrated via drinking water to induce depression- like behavior in a 30-days period. Liraglutide with different dosages were started to treat the depression model from the 15th day to the end. (B) Forced swim test (FST). In FST, the CORT group showed decreased mobility time in comparison with control (one-way ANOVA, ***: $p < 0.001$). Compared with the CORT group, liraglutide at the higher dosage (20nm/kg) increased the mobility time (one-way ANOVA, ###: $p < 0.001$). The positive control fluoxetine (FLX) also showed the same effects in FST (one-way ANOVA, ###: $p < 0.001$ in compare with CORT). (C) Tail suspension test (TST). In the TST, the CORT group showed significant decreased mobility time in comparing with control (one-way ANOVA, ***: $p < 0.001$). Compared with CORT group, liraglutide at the higher dosage (20nm/kg) increased the mobility time (one-way ANOVA, #: $p < 0.05$). FLX showed similar effects (one-way ANOVA, ##: $p < 0.01$ in compare with CORT). (D) Elevated plus maze (EPS) test. Compared with control, CORT treatment decreased the time in the open arm (one-way ANOVA, ***: $p < 0.001$). FLX treatment increased the exploration in the open arm (one-way ANOVA, ###: $p < 0.01$ compared with CORT). (E) Open field test (OFT). Compared with control, CORT decreased exploration time in the central region of the open field (one-way ANOVA, ***: $p < 0.001$). Both low and higher doses of liraglutide increased the exploration time (one-way ANOVA, #: $p < 0.05$ #: $p < 0.01$ compared with CORT). FLX treatment showed similar effects (one-way ANOVA, #: $p < 0.05$ in compare with CORT). (n=12 per group)

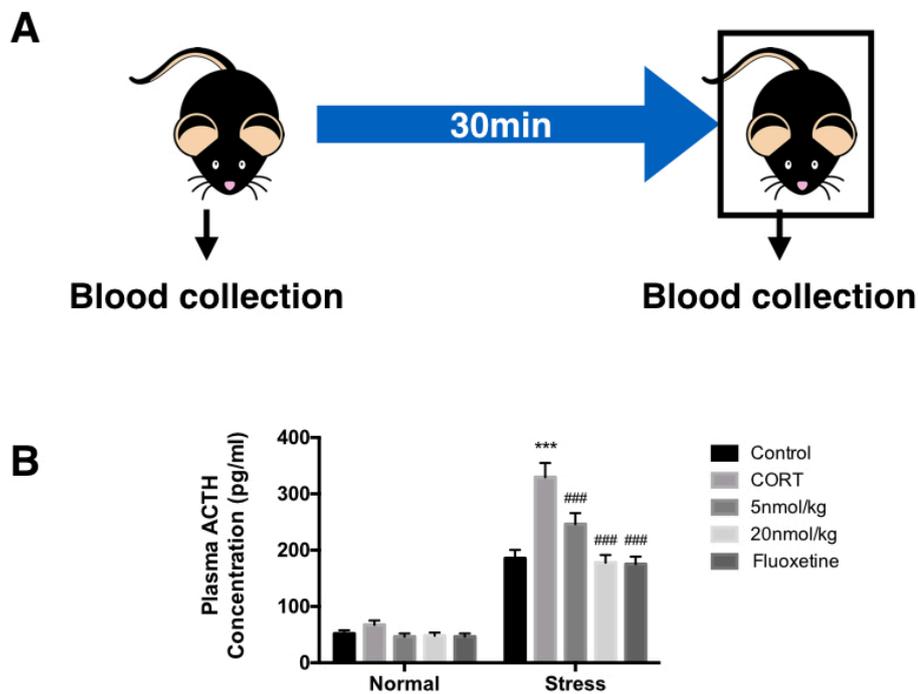


Figure 2: Enhanced ACTH levels in the blood are reduced by liraglutide treatment

(A) Experimental protocol to test the adreno-cortico-tropic-hormone (ACTH) of adult mice. Mice in different groups were collected with blood at normal condition. Then after underwent the 30min restraint stress, the blood was collected again to prepare the plasma sample. (B) Statistical analysis indicates that no different level of ACTH showed among different groups under normal condition. Although restraint induced the increasing ACTH in plasma, CORT administrated group presented the higher ACTH level at stress condition compared with control (two-way ANOVA, $F(4, 50) = 9.593$, $p < 0.0001$; CORT vs control under stress ***: $p < 0.001$). In addition, different dosages of liraglutide reduced the CORT induced hyper-elevation of ACTH under stress, which had same effect with fluoxetine (two-way ANOVA, under stress, compare with CORT group ####: $p < 0.001$). $N=6$ per group.

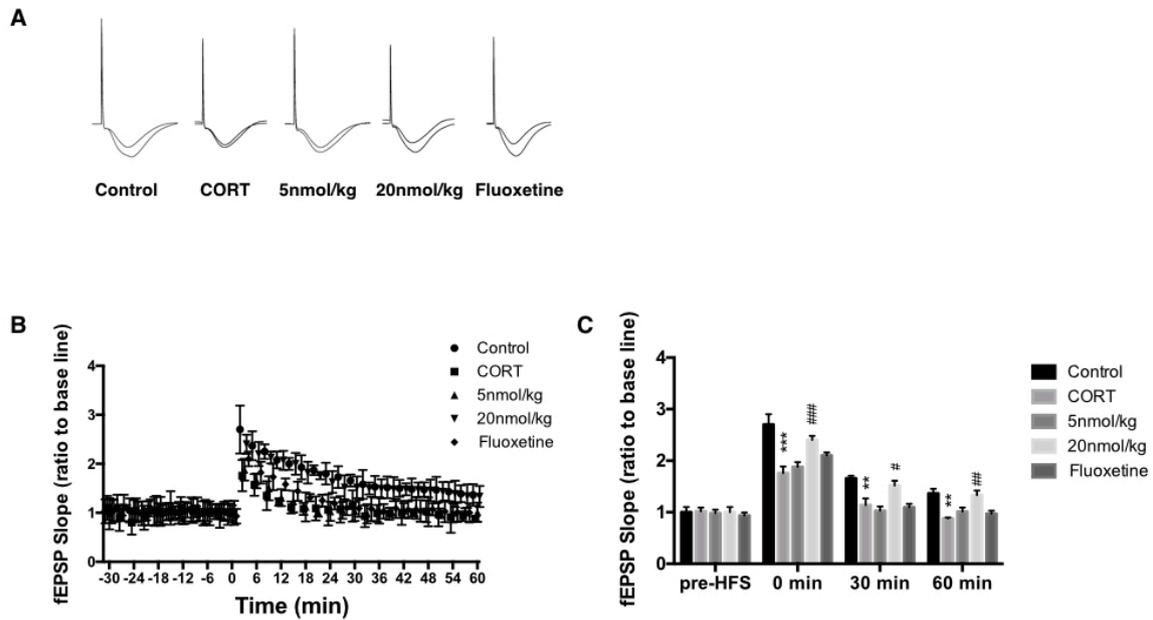


Figure 3: Liraglutide prevented the CORT induced impairment of hippocampal LTP

(A) Representative fEPSP traces recorded prior and after HFS. (B) fEPSP slopes before HFS and after HFS for each group. (C) Statistical analysis to compare the fEPSP slope values of different groups at different time point after HFS. CORT administration decreased fEPSPs at all three time points (0, 30min and 60min) (two-way ANOVA, ***: $p < 0.001$ in compared with control). The high dosage of liraglutide (20nm/kg) reversed this effects. In compared with CORT, the high dose of liraglutide increased the slopes of fEPSP at three time points (two-way ANOVA, ###: $p < 0.001$, ##: $p < 0.01$, #: $p < 0.05$ compared with CORT). However, treatment with FLX did not show any effects. N=6 per group.

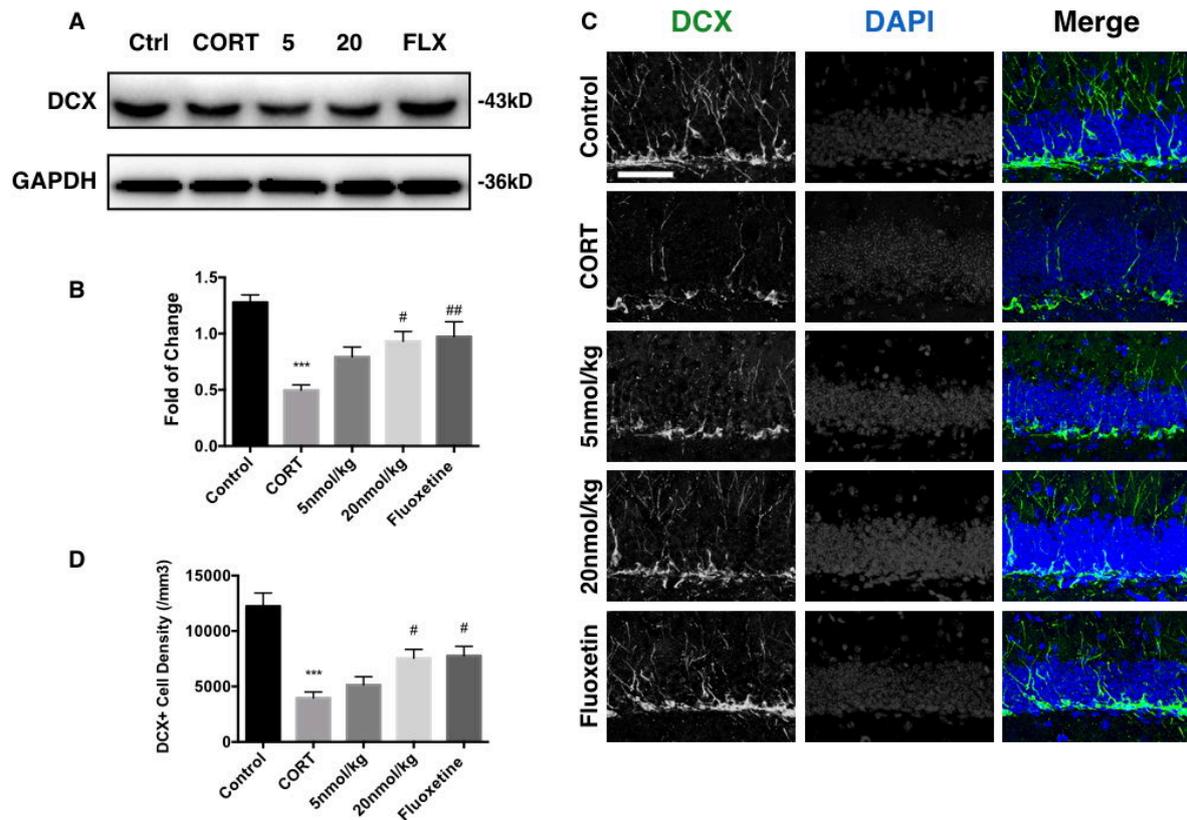


Figure 4: Liraglutide promotes adult neurogenesis in the hippocampus

(A) Western blot bands to show the levels of doublecortin (DCX). (B) DCX levels in the 5 groups. CORT induced a decrease of DCX in the hippocampus (one-way ANOVA, ***: $p < 0.001$ compared with control). The high dosages of liraglutide treatment elevated the DCX levels (one-way ANOVA, #: $p < 0.05$ compared with CORT). FLX treatment enhanced DCX levels (one-way ANOVA, ##: $p < 0.01$ compared with CORT). (C) Immunofluorescence sample image of DCX+ immature neurons in the DG (bar=50 μ m). (D) Cell density of DCX+ immature neurons in the DG region. CORT treatment significantly reduced the density of immature neurons (one-way ANOVA, ***: $p < 0.001$ compared with control). The high dose of liraglutide increased the immature neuron density (one-way ANOVA, #: $p < 0.05$ compare with CORT). FLX increased the levels of DCX+ neurons (one-way ANOVA, #: $p < 0.05$ compared with CORT). (n=6 per group).

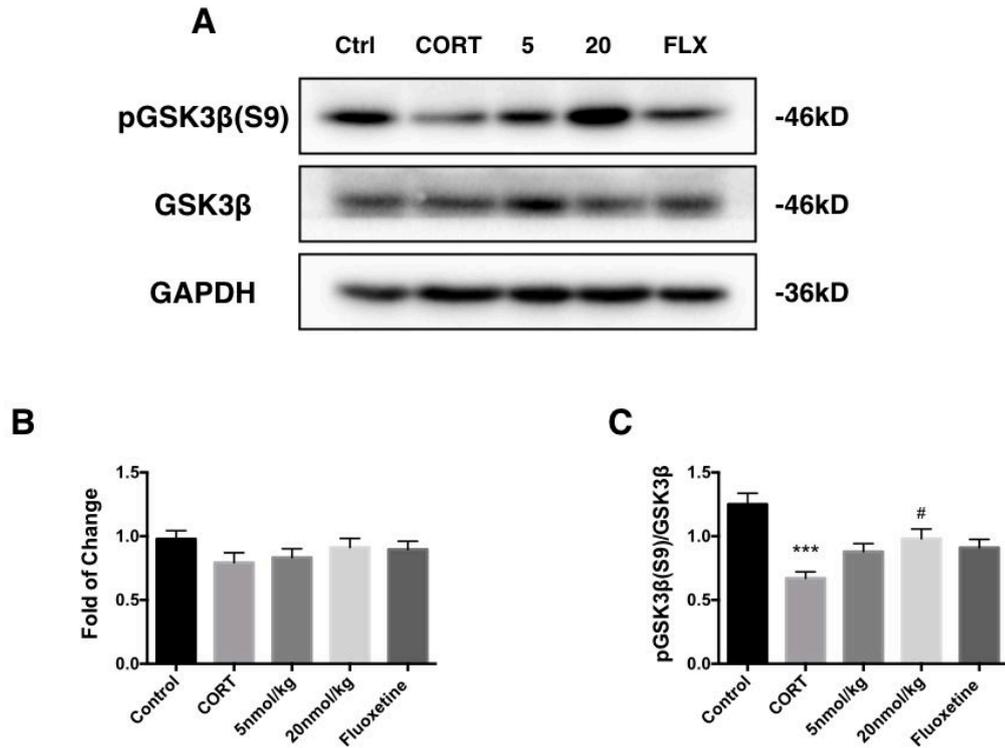


Figure 5: Liraglutide inhibits the activity of GSK3 β by regulating the phosphorylation level

(A) Western blot bands to show the phosphorylation of GSK3 β at Ser9 and the total GSK3 β levels (B) Levels of GSK3 β total protein. One-way ANOVA showed there is no difference among all groups in the total protein levels. (C) Phosphorylation level of GSK3 β at Ser9. CORT administration resulted in a decreased phosphorylation of GSK3 β at Ser9 (one-way ANOVA, ***: $p < 0.001$, compared with control). In compared with CORT, treatment of liraglutide with high dosage increased GSK3 β phosphorylation at Ser9 (one-way ANOVA, #: $p < 0.05$). In the FLX group, there was no increase of GSK3 β phosphorylation at Ser9. (n=6) per group.